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3203-Plat
The Molecular Structure of the Liquid Ordered Phase
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Substructure within the liquid-ordered phase of lipid bilayers is reported, composed of saturated hydrocarbon chains packed with local hexagonal order, separated by interstitial regions enriched in cholesterol and unsaturated chains. The structure, identified during 10 μsec all-atom molecular dynamics simulation of liquid-ordered/liquid-disordered coexistence (Lo/Ld), is confirmed by comparison to 2H NMR quadropolar splittings. The balance of cholesterol-rich to local hexagonal order is proposed to control the partitioning of membrane components into the Lo region. Understanding how mechanisms present in the cell membrane _ such as crowding or coupling to the cytoskeleton _ shift the balance of cholesterol rich substructure should help resolve partitioning in bilayers and cell membranes.

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A Lipid Bound Actin Meshwork Organizes Liquid Phase Separation in Model Membranes
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The cytoplasmic side of eukaryotic cell membranes is covered by a dense actin rich cortex. We present FCS and STED experiments showing that a dense membrane bound actin network has severe influence on temperature dependent lipid phase separation. A minimal actin cortex was bound to a supported lipid bilayer via biotinylated lipid streptavidin complexes (pinning sites). In general, actin binding to ternary membranes prevented macroscopic liquid-ordered (Lo) and liquid-disordered (Ld) domain formation in these systems, even at low temperature. For pinning sites that strongly attract Ld domains, an actin correlated multi-domain pattern was observed, consisting of Ld ''channels'' along the actin fibers, with Lo ''islands'' in the voids. FCS measurements revealed enhanced diffusion of unsaturated lipids along the channels, and hindered diffusion of these lipids in directions perpendicular. For pinning sites strongly attractive to Lo domains, an ''inverse'' domain structure was observed. These findings are in good agreement with a number of recently proposed simulation models. However, to fully capture our experimental observations, an extended simulation model is proposed, in which the lipid domains also couple to the local membrane curvature. Our results provide a mechanism how cells may prevent macroscopic de-mixing of membrane components and at the same time regulate the local membrane compositions.

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The Impact of Oxidized Phospholipids on Lipid Membranes: Consequences for Mitochondrial Apoptosis
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Mitochondria play a crucial role in the intrinsic apoptotic pathway. Key regulators of this pathway are the Bcl-2 family proteins who interact with the mitochondrial outer membrane to modulate membrane permeability. Intracellular oxidative stress is one major factor leading to apoptosis accompanied by the permeabilization of the mitochondrial outer membrane; a process causing release of apoptotic factors such as cytochrome c. Upon onset of intracellular stress phospholipids can become oxidized. These oxidized phospholipids (OxPls) can severely alter the properties of these mitochondrial membranes, and can therefore have i) a direct effect on the membrane properties and its perforation and ii) can have an indirect effect by altering the function of membrane-coupled Bcl-2 proteins (such as the anti-apoptotic Bcl-2 or the apoptotic Bax). We have been using either of the oxidized phospholipids (OxPls) PoxnoPC and PazePC, with an aldehyde or a carboxyl group respectively at their truncated *sn*-2-chain ends. Based on earlier DSC and ^{31}P ssNMR studies of DMPC bilayers doped with either PoxnoPC or PazePC, we produced now mitochondrial outer membrane mimicking bilayers composed of POPC/POPE/TMCL where we simulated oxidative stress by incorporation of OxPls. Differential scanning calorimetry and ^{1}H, ^{13}C- and ^{31}P MAS NMR at different temperatures were used to further describe the organization of these membranes and their OxPls dependent affinity to the apoptotic Bax protein, which in apoptosis becomes targeted to the mitochondria where it induces permeabilization. In addition, novel fluorescence leakage assays are used to study the influence of the presence and type of OxPls on the membrane permeabilization ability of Bax protein using giant (GUVs) and large (LUVs) unilamellar vesicles.

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G-Protein-Coupled Receptor Activation Investigated using Small-Angle Neutron Scattering
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G-protein-coupled receptors (GPCRs) represent the largest family of proteins in the human genome and comprise about 50% of current molecular drug targets. Rhodopsin is the GPCR involved in visual light perception and occurs naturally in a membrane lipid environment. Rhodopsin photoactivation yields *cis-trans* isomerization of retinal giving an equilibrium between inactive Meta-I and active Meta-II states. Here we address the question: does photoactivation lead to a single Meta-II conformation or is there an ensemble of substates described by an ensemble-activation mechanism (EAM) [1]? In this context small-angle neutron scattering (SANS) probes rhodopsin-detergent and rhodopsin-lipid complexes through measurement of the intensity of the neutrons scattered as a function of scattering vector $I(q)$. Contrast variation enables us to highlight individual components of a multi-component system without isotopic labeling of the sample. Upon photoactivation, the Meta-I state was stabilized in CHAPS-solubilized rhodopsin, while Meta-II was trapped in DDM-solubilized rhodopsin. The ligand-free apoprotein opsin was obtained by photobleaching rhodopsin in the presence of hydroxylamine. The SANS spectra for the above rhodopsin substates were acquired from 80% D_2O solutions and at contrast-matching points for both DDM and CHAPS samples. The data collected in 80% D_2O samples provide structural information for both protein and detergent, while the data collected at contrast-matching points give information for the protein structure exclusively. Our experiments demonstrate that for detergent-solubilized rhodopsin, SANS with contrast variation can detect structural differences between the rhodopsin dark-state, Meta-I, Meta-II, and ligand-free opsin states. Dark-state rhodopsin is more conformationally flexible (less-compact) in DDM micelles compared to the CHAPS, which is consistent with an ensemble of activated Meta-II states. Furthermore, the time-dependent structural transitions between Meta-I and Meta-II as observed by time-resolved SANS will be crucial to understanding the ensemble-based activation. [1]A.V. Struts *et al.* (2011) *NSMB***18**, 392-394.

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MemProtMD: Membrane Protein Structures and Simulations
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Membrane protein structural biology is one of the key biochemical challenges of the coming decade. The perpetual enhancements to the methods used to acquire novel structures have led to an exponential growth in the number of membrane proteins visualised at high-resolution. Nevertheless, these biological assemblies are usually captured in the absence of their native lipid environment. Coarse-Grained molecular dynamics (CGMD) simulations provide a means for assessing the assembly and interactions of molecular complexes at a reduced level of representation. This method has been shown to be very accurate in predicting the orientation and interactions of proteins within explicit lipid membranes. We have automated the methodology, so that new protein structures are automatically detected, through a set of novel algorithms, and inserted into a cell membrane upon their release in the Protein Data Bank (PDB). The resultant simulations are then assessed for protein-lipid interactions, bilayer deformation, lipid diffusion, protein tilt and small molecule access pathways. The complexes are then transformed to better reflect the native membrane composition and also converted to an atomistic resolution to investigate the dynamics in full atomic detail. The resulting analysis and structure files are then made accessible from our MemProtMD website: http://sbcb.bioch.ox.ac.uk/memprotmd/. In addition to the automated pipeline, we have applied this methodology to a number of recently resolved membrane protein structures to assess their location within the membrane, structural stability and to identify specific lipid binding sites (1,2).
1) Quigley et al. Science (2013) 339:1604-7.
2) Rollauer et al. Nature (2012) 492:210-4.